DATA EVALUATION RECORD
FISH FULL LIFE-STAGE TEST
GUIDELINE 72-5

1 CHEMICAL: Diclofop-methyl
Shaughnessey No. 110902

2. TEST MATERIAL: Technical: methyl 2-[4-(2,4-dichlorophenoxy)phenoxy]propanoate Purity: 95.3%

3. CITATION: Dionne, E. 1994. Diclofop-methyl (Hoe 023408 00 ZD95 0004) the Chronic Toxicity to the Fathead Minnow (Pimephales promelas) During a Full Life-Cycle Exposure. SLI Study #1719.0692.6199.122; SLI Report #94-3-5180. Prepared by Springborn Laboratories, Inc. Environmental Sciences Division; Wareham, MA; Submitted by Hoechst Celanese Corp., Somerville, NJ.; MRID No. 43284601.

4. REVIEWED BY:

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5. APPROVED BY:

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6. CONCLUSIONS: This study is scientifically sound and satisfies the guideline requirement for a fish full life-cycle
study. The MATC of diclofop-methyl equivalents for fathead minnow is 10.6 ug/L (NOEC = 7.5 ug/l and LOEC = 15 ug/L) based upon reduced larval growth.

7. ADEQUACY OF THE STUDY:

A. Classification: Core

B. Rationale: N/A

C. Reparability: N/A

8. GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

A GLP statement was included in the report indicating the study followed GLP procedures except that routine water and food contaminant screening analyses for pesticides, PCB and metals were conducted using standard U.S. EPA procedures, rather than GLP procedures.

9. MATERIALS AND METHODS:

A. Biological System:

Guideline Criteria	Reported Information
Species: preferred species are fathead minnow (Pimephales promelas) and sheepshead minnow (Cyprinodon variegatus)	test species is the fathead minnow (Pimephales promelas)
Source	fish were obtained from Springborn's culture unit
Age at beginning of test: embryos 2 to 24 hours old	naturally fertilized fathead minnow eggs were <24 hours old at test initiation
Replicates: minimum of 50 embryos (≤24 hrs old) per replicate cup, 4 replicates per concentration	50 embryos per replicate cup; two cups per aquaria; two aquaria per treatment level; embryos were randomly assigned
Larval-juvenile exposure- minimum of 25 fish per replicate larval growth chamber	25 newly hatched larvae were impartially selected from each cup and placed in larval growth chambers (2/aquarium); 2 aquarium per treatment level
Juvenile-adult exposure- mature fish are placed in a spawning tank (separated into 4 individual spawning substrates); 4 males and 4 females randomly chosen and assigned	3 compartments per aquarium- allowing 3 separate spawning groups per aquarium (2 aquarium per treatment level); spawning group consisted of 1 male and 2 females (not indicated in report if randomly chosen)

Guideline Criteria	Reported Information
Post Hatch: % of embryos that produce live fry must be $\geq$ 50% in each control; % hatch in any control embryo cup must be no more than 1.6 times that in another control cup	per cent hatch was good, averaged 93.1% in controls
Feeding: fish should be fed at least twice daily. Fish should not be fed for at least 24 hr prior to termination on day 32	newly hatched fry were fed live brine shrimp nauplii 3 times daily for 1st 30 days; next 30 days fish were fed 2 times daily (one feeding frozen brine shrimp, other Zeigler Brothers Prime flakes); weekend feedings were one less each day
Measurements/Counts- embryo exposure period	until hatch began on day 4, embryos were counted and dead ones were discarded; on day 4, embryos and hatched larvae were observed and dead embryos were discarded; % hatch was based upon # of embryos at initiation (100/replicate aquarium); if there were 2 or more unaccounted for in a cup, the % hatching success was based upon #live fry/#organisms
Measurements/Counts- larval-juvenile exposure period 8 wks)	25 hatched larvae were impartially selected and placed into larval growth chambers (2 growth chambers per aquarium/ 2 aquarium per treatment), larvae which were unaccounted for were considered mortalities; at 30 days post-hatch the total length (photographed over grid) was determined; at 59 days 25 larvae from each rep were randomly selected to remain in exposure and were photographed to determine length; survival was calculated

Guideline Criteria	Reported Information
Measurements/Counts- Juvenile-adults exposure period	at 90 days post-hatch total length was determined; at 160 days post-hatch territorial males were observed in all test aquaria - spawning groups (1 male/2 female each) were transferred to lower aquarium; records were maintained for each spawning group and included # eggs spawned and # eggs incubated; 50 eggs from the ist 10 spawns of $\geq$50 eggs in each aquarium were incubated for % hatch determination; after that every 3rd spawn of $\geq$50 eggs were incubated for % hatch determination; exposure of the F_0 parental fish was terminated after 265 days of exposure- individuals were examined to verify sex and gonadal condition, deformities, injuries, and total length and wet weight
Measurements/Counts- second generation embryo exposure (45 days); larvae should have been produced from different breeding pairs in each tank	as embryo groups hatched, groups of 25 were established in each aquarium (groups were established with progeny from 2 different pairs of adult F_0 spawners); after 30 days post-hatch F_1 larval groups were terminated and the % survival for each group was calculated, individual lengths and wet weights were determined
Controls: average survival at end of test must be $\geq$ 80%. Survival in any control chamber must not be $<$70%	average. survival in controls was good and averaged $\geq$90%
Controls: negative control and carrier control (when applicable) are required	test included both negative and solvent controls

B. Physical System:

Guideline Criteria	Reported Information
Test Water: 1) may be natural (sterilized and filtered) or a commercial mixture; 2) natural seawater should have weekly range of salinity less than 6%, monthly pH range less than 0.8 pH units; 3) salinity should be $\geq$ 15 parts per thousand; 4) water must be free of pollutants	dilution water was drawn from a 125 m deep bedrock well into a concrete reservoir where it was aerated and supplemented with well water from Town of Wareham, MA; semi-annual chemical analyses for presence of pesticides, PCB's, and toxic metals showed no contaminants at levels high enough to be considered toxic; total organic carbon concentration ranged 0.44 to 1.4 mg/L during exposure based on monthly analysis
Test Temperature: depends upon test species; should not deviate by more than 2°C from appropriate temperature. For sheepshead minnow, either 25°C or 30°C is recommended	temperature of the water in the culture tanks was 24 $\pm$ 1°C.; temperature of the exposure solutions was maintained at 25 $\pm$ 1°C. by heated water baths; incoming dilution water was preheated by gas-fired glass-lined water heaters before flowing through aged PVC piping to the test system temperature was measured daily in one replicate aquarium of each treatment level and the controls; temperature was continuously monitored in one aquarium on each level of the diluter system using a min/max thermometer
Photoperiod: recommend 16L/8D	a graduated photoperiod was used and was based on dusk to dawn times in Evansville, Indiana; illumination range was 60 to 150 footcandles at the solution surfaces- lighting was by fluorescent bulbs; report did not indicate if there were gradual dawn/dusk simulations
Concentrations with a dilution factor not greater than 0.5 and controls should be used	intermittent flow proportional diluter (Mount and Brungs, 1967); five toxicant concentrations, plus control and solvent control (acetone); dilution factor of 5 was used; acetone amount of 0.0087 mL/L was present in the highest concentration

Guideline Criteria	Reported Information
Toxicant Mixing: 1) mixing chamber is recommended but not required; 2) aeration should not be used for mixing; 3) it must be demonstrated that the test solution is completely mixed before intro. into the test system; 4) flow splitting accuracy must be within 10%	mixing chamber was employed; solution was continuously stirred using a magnetic stirrer and Teflon coated stir bar; flow-splitting chambers were employed; flow-splitting accuracy was not reported
Exposure System/Test Vessels: all glass or glass with stainless steel frame	glass aquaria with silicone sealant seams and equipped with a 15 cm high end-drain to maintain test solution volume at 27 liters were used; in the upper level there were 2 larval growth chambers (30 cm x 13 cm x 25 cm) in each aquarium; in the lower level each aquarium was divided into 3 compartments using nylon mesh screen dividers; spawning sites were made from 10 cm sections of PVC pipe, 4" diameter which were halved and place concave surface down; two substrates were provided to each spawning group
Embryo Cups: 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen	at inflow of each aquarium a 7.5 cm x 16 cm x 7.5 cm incubation chamber was attached to which two embryo cups were placed; cups were 5 cm diameter glass jars with nylon screen bottoms
Flow Rate: flow rates to larval cups should provide 90% replacement in 8-12 hours. Flow rate must maintain DO at above 75% of saturation and maintain the toxicant level	there were 18 volume replacements every 24 hrs (90% replacement time of 2.5 hrs); during the spawning phase average turnover rate was 14 volume replacements daily (or 90% replacement in 3.5 hrs); DO was maintained at >75% saturation
Aeration: dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated	test tanks and embryo cups were not aerated

C. Chemical System:

Guideline Criteria	Reported Information
Concentrations: minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate. - toxicant conc. must be measured in one tank at each toxicant level every week	- five concentrations plus control and solvent control, each replicated twice - toxicant conc. measured in all tanks on test days 0, 8, 15, 22, 29 and 37
Analytical methodology	test solution in each aquarium was sampled a minimum of once per wk until spawning, after which samples were taken weekly from one replicate of each treatment level and the controls; samples were removed from alternating replicates weekly; high performance liquid chromatographic procedure used; tissue representing major life stages of the fathead minnow (pre-spawn fish, post-spawn fish, <24-hr old F_1 embryos and 30-day old larvae were also analyzed
Other Variables: 1) DO must be measured at each conc. at least once a week; 2) natural seawater must maintain a constant salinity and not fluctuate more than 6% weekly; monthly pH range < 0.8 pH units	DO and pH were measured daily in one replicate aquarium of each treatment level and the controls; total hardness, total alkalinity and specific conductivity were measured weekly in one control (dilution water or solvent) and one aquarium on a rotating basis
Solvents: should not exceed 0.1 ml/L in a flow-through system. Following solvents are acceptable: dimethylformamide, triethylene glycol, methanol, acetone, ethanol	solvent control was equal to the concentration of solvent in highest treatment level (0.0087 mL/L acetone)

Statistical Design: Statistical analyses of continuous data (growth and reproduction) were performed using mean responses from each of the two replicate aquaria. Data for solvent control and dilution water control were compared using Student's T-test. If no statistical difference, data were pooled, otherwise solvent control data were used for comparison to treatment levels. William's test was used for statistical comparisons. Survival/hatching success

were conducted using a appropriate contingency table test.

10. REPORTED RESULTS:

Analytical Results: Stock solution was renewed monthly based on stability testing which showed that methyl concentrations measured varied minimally (mean 93.3%) of nominal, and because degradation was not observed after 32 days of aging. The mean measured concentrations as methyl equivalents, maintained during the 265-day test, were 3.8, 7.5, 15, 31, and 63 ug/L. These averaged 61% of the nominal concentrations.

Pre-exposure solution analyses were performed and an effort was made to maximize the amount of test material solubilized and measured in the exposure solutions (59% of the nominal concentration was achieved). A summary of findings: (1) volatilization was ruled out, (2) increasing the temperature of the dilution water or amount of stock solution added during each cycle did not work, (3) no trend for adsorption of test material to glass surfaces was observed, (4) no conclusive evidence of test material degradation in the delivery system was obtained, and (5) the concentration of ^{14}C residues in an exposure solution sample was 8% higher than the concentration as methyl equivalents determined by HPLC-UV, and based on the indication of the presence of a degradate additional to diclofop acid, GC-MS analysis was conducted and a peak was identified as 1,2 benzenedicarboxylic acid.

Water Quality Measurements: Water quality measurements are provided in the attached Table 5. Total hardness, total alkalinity, specific conductance, dissolved oxygen, pH and temperature were found to vary minimally throughout the study. Dissolved oxygen concentrations dipped below 75% on certain occasions (Table 5, attached), but were back in range within 48 hours.

Reported Statistical Results for Biological Endpoints:

Guideline Criteria	Reported Information
Data Endpoints must include: - survival of F_0 and F_1 embryos, time required to hatch, hatching success, and survival of fry - survival of F_0 fish during larval-juvenile exposure period - at four and eight weeks after hatching, total lengths of fish - at eight weeks after hatching of F_1 fish, weights and lengths are recorded - incidence of pathological or histological effects - observations of other effects or clinical signs	Endpoints included: F_0 embryo hatching success F_0 embryo larval survival after 30, 59, and 265 days F_0 embryo larval growth (length) after 30 days F_0 embryo larval growth (length and wet weight) after 59 and 265 days F_0 embryo larval growth (length) after 90 days F_1 embryo hatching success and time-to-hatch F_1 larval 30-day survival F_1 larval 30-day growth (total length and wet weight) also measured were: total #spawns, total #eggs, #eggs/spawn, #spawns/female and #eggs/female results are contained in Tables 9 through 14, attached

Statistical analysis for F_0 endpoints showed no significant difference between solvent control and dilution water control, except for 30 and 59 day survival parameters. Data for controls were pooled in all cases, except for the later (comparisons made only to solvent control).

F_0 30 and 59 day post-hatch survival was significantly reduced at 63 ug/L. After 30 days of exposure, length of fish exposed to 15 and 31 ug/L concentration was significantly reduced (data at the 63 ug/L test concentration was not analyzed because of the significantly reduced survival at this test concentration). After 59 days of exposure, all groups were significantly reduced (the study authors considered this an apparent effect on length, since no statistical significant differences in weight). After 90 days, total length in all test concentrations were comparable to the pooled control. Reproductive success was unaffected at all test concentrations. Survival and growth at test termination (265 days) was unaffected at all test concentrations.

The hatching success of F_1 fry was significantly reduced at test concentration levels 31 and 63 ug/L. At 30-days post-hatch, survival at the 62 ug/L was significantly reduced, and also at the 15 ug/L level (the study authors considered this finding to be not toxicant-related, since no statistically significant reduction occurred at the 31 ug/L test concentration level). There were no statistically significant reductions in length and weight of fish at $\leq$31 ug/L (data at the 63 ug/L test concentration was not analyzed because of the significantly reduced survival at this test concentration). The most sensitive indicator of toxicity was the reduced hatching success of the F_1 embryos. The effects observed on length were considered transitory, since they were only observed on test days 30 and 59, not at 265 days (study termination). The MATC of diclofop-methyl equivalents for fathead minnows was estimated to be >15 ug/L (NOEC) and less than 31 ug/L (LOEC). The geometric mean was calculated to be 22 ug/L.

11. Reviewer's Statistical Results:

Statistical Method: Williams Test (see attached)

Biological EndPoint	NOEC (ug/L)	LOEC (ug/L)
F_0 embryo larval survival (30 days)	31	63
F_0 embryo larval growth (length at 30 days)	15	31
F_0 embryo larval survival (59 days)	31	63
F_0 embryo larval growth (length at 59 days)	7.5	15
F_0 embryo larval growth (weight at 59 days)	15	31
F_0 embryo larval growth (length at 90 days)	7.5	15
F_1 embryo larval survival (30 days)	31	63

Most Sensitive Endpoint:

F_0 embryo larval growth (length at 59 days) and F_0 embryo larval growth (length at 90 days); NOEC = 7.5 ug/L and LOEC = 15 ug/L. The MATC is 10.6 ug/L. The EEB does not agree with the study author that the effects observed on length at 59 and 90 days should be considered transitory. It is conceivable (and therefore should not be ruled out) that there could be an effect on survival of a fish population if these fish are already under stressed environmental conditions (e.g. lack of food).

Water Quality Measurements: Measurements of total hardness, total alkalinity, specific conductance, dissolved oxygen, pH and temperature appeared to be within acceptable ranges for the survival, growth and reproduction of the fish.

Analytical Results: Although mean measured concentrations only averaged 61% of the nominal concentrations, this does not detract from the quality of the study because the MATC is derived from the more conservative mean measured concentrations.

Conclusions: This study is scientifically sound and satisfies the guideline requirement for a fish full life-cycle study. The MATC of diclofop-methyl equivalents is 10.6 ug/L (NOEC = 7.5 ug/l and LOEC = 15 ug/L) based upon reduced larval growth.

12. COMPLETION OF ONE-LINER FOR STUDY: